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We have taken a modular approach to design peptides which adopt defined structures and perform specific tasks. Peptides are being designed to bind either a specific sequence of double stranded DNA or the pp56^{lck} tyrosine protein kinase which likely is the cytoplasmic effector of a transmembrane receptor whose activation *in vivo* is associated with an increase in cytoplasmic free calcium. Peptides have been synthesized which bind to each of these macromolecules with K_{DS} near 2μM. In addition to binding pp56^{lck} tightly, one peptide has been found to stimulate the activity of this enzyme up to 20-fold. Initial results suggest that ionic interactions are important for this activation. Currently work is continuing on both projects. DNA binding peptides are being redesigned to maximize their ability to discriminate between different nucleotide sequences. pp56^{lck} binding peptides are being studied to enable modeling signal transduction across membranes.

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PRINCIPAL INVESTIGATOR: H. Neal Bramson

CO-INVESTIGATORS: Lisa Regan and Chris Sommers

CONTRACTOR: The University of Rochester

CONTRACT TITLE: A Modular Approach to Protein Design

START DATE: 1 February 1988

RESEARCH OBJECTIVES:

A modular approach for protein design, which utilizes segments of protein structures as modules to construct hybrid proteins with new specificities, has been taken to study macromolecular interactions. In this way DNA-protein interactions are being studied through incorporating the helix-turn-helix of the DNA binding *lac* repressor into ribonuclease A to transform this protein into one which binds double stranded DNA. Similarly, in order to study protein-protein interactions a peptide incorporating a region of the CD4 receptor has been synthesized and shown to activate pp56^{lck}, the physiological ligand of the CD4 receptor, 20-fold. The long term goal of the pp56^{lck} work is to attach a regulatory module to this activating peptide and make this activation responsive to small molecules.

PROGRESS (YEAR 2):

DNA BINDING MODULES

The sequence of the first DNA binding module we synthesized is:

```
                5                   10                  15
Met-Lys-Pro-Val-Thr-Leu-Tyr-Glu-Gln-Ala-Glu-Gln-Ala-Gly-Val-
                20                  25                  30
Ser-Tyr-Val-Gln-Val-Ser-Arg-Gln-Val-Asn-Ala-Ala-Ser-Lys-Phe-
                35
Glu-Arg-Gln-His-Met-Asp-Ser
```

PEPTIDE 1

Peptide 1 is comprised of *lac* repressor residues 1-28 fused to ribonuclease residues 7-15 with several amino acid residue changes made to increase the stability of peptide structure. As expected, peptide 1 binds tightly to the ribonuclease S protein (K_D=3μM). A CD analysis of peptide 1 in the presence and absence of the S protein indicates that the complexed peptides are substantially more helical than the free peptides. It appears therefore that at least some of the desired peptide structure (such as the *lac* repressor helix-turn-helix) is present in the complex. To test DNA binding by this complex, peptide 1 and ribonuclease S protein were incubated together with a 180bp PUC18 fragment which contains the *lac* operator. The extent of

DNA binding was determined by gel shift assays and DNAase footprinting. No specific interactions were detected. At concentrations above 50μM, complex binds to the DNA in a nonspecific fashion which can be competed off with calf thymus DNA or high concentrations of salt.

The next two peptide modules retained more alanine residues to stabilize the helices and additional *lac* repressor residues:

```
                         5                   10                  15
Met-Lys-Lys-Val-Thr-Leu-Tyr-Glu-Ala-Ala-Glu-Ala-Ala-Gly-Val-
                        20                   25                  30
Ser-Tyr-Gln-Thr-Val-Ser-Arg-Lys-Val-Ala-Ala-Ala-Ala-Lys-Phe-
                        35
Glu-Arg-Gln-His-Met-Asp-Ser
```

Peptide 2

with peptide 3 containing an asparagine, such as is found in the *lac* repressor, at residue 25 in place of alanine. Each peptide appears to bind the ribonuclease S protein approximately as well as does peptide 1. However, as part of the complex with the S protein, peptides 2 and 3 each bind to a 140bp fragment which contains the *lac* operator at least 10-fold more tightly than the complex containing peptide 1. Even at concentrations of about 5μM of complex DNA binding is efficient and not inhibited by 0.25M concentrations of salt. Higher salt concentrations inhibited not only this interaction but also substantially decreased DNA binding by the native *lac* repressor. Complexes containing peptides 2 and 3 also bind tightly to sequences that do not contain the *lac* operator, including other PUC18 fragments and calf thymus DNA. At this time the ratio of specific to nonspecific binding is not known and is currently being investigated.

To facilitate comparisons between specific and nonspecific DNA binding we have synthesized a palindromic oligonucleotide which self anneals to form a strong *lac* repressor binding site:

TTAATTGTGAGCGCTCACAATT

We have also synthesized a 51 amino acid residue fragment of the *lac* repressor referred to as the *lac* headpiece to characterize peptide binding to the oligonucleotide. The *lac* headpiece has been shown in a number of laboratories to bind to sequences similar to the above oligonucleotide. We have used filter binding assays to measure the association of oligonucleotide and the headpiece. The effect of dimerization, which might be useful for increasing the DNA binding affinities of peptide modules, on *lac* headpiece DNA binding, was also investigated. Initial results suggest that monomeric *lac* headpiece binding to the above oligonucleotide is characterized by a K_D of about 3μM and that when this peptide is dimerized through oxidation of a cysteine residue included on the carboxy terminus of the headpiece this binding interaction seems to be about 3-fold tighter. Work is continuing to investigate the effect of dimerization on peptide binding to palindromic DNA sites.

PROTEIN-PROTEIN INTERACTIONS

Cytoplasmic tyrosine protein kinases of the *src* family are protooncogene products that are thought likely to play crucial roles in signal transduction through cellular receptors. One of these enzymes, pp56^{lck}, is associated with the transmembrane receptor CD4 in CD4^{+} T cells. There is now substantial evidence that the 38 amino acid residue cytoplasmic domain of this receptor regulates the activity of the protein kinase through protein-protein interactions. The goal of this project is to elucidate the mechanism for this regulation to enable the synthesis of peptide models which reversibly regulate enzymic activities.

An examination of the CD4 and pp56^{lck} protein sequences deposited in GenBank indicate that, assuming histidine is uncharged at pH 7.4, the cytoplasmic portion of murine CD4 has a net charge of +8 while that of murine pp56^{lck} has a net charge of -19. Reasoning that ionic interactions might play a role in the associations of CD4 and pp56^{lck} or in the regulation of enzymic activities, we synthesized the following peptide which reproduces the sequence of a heavily charged region in the cytoplasmic domain of murine CD4 and contains an additional tyrosine residue for future iodinations:

5 10
Acetyl-Tyr-Val-Arg-Cys-Arg-His-Gln-Gln-Arg-Gln-Ala-Ala-Arg-Met-
15 20 25 30
Ser-Gln-Ile-Lys-Arg-Leu-Leu-Ser-Glu-Lys-Lys-Thr-Cys-Gln-Cys-Pro-

His-Arg-Met

ACTIVATOR 1

Activator 1 stimulates the phosphotransferase activities of the pp56^{lck} protein kinase up to 20-fold in a dose dependent manner that is described by an apparent K_D of about 2μM. In contrast, the addition of millimolar concentrations of the heptapeptide comprised of activator 1 residues 3-9 or the hexapeptide $(Lys)_6$ do not alter pp56^{lck} activities. The specificity of interactions between activator 1 and pp56^{lck} were further demonstrated by the finding that activator 1 has no effect on the activities of the highly homologous pp60^{c-src} and pp60^{v-src} protein kinases.

If ionic attractions are important for effective interactions between activator 1 and pp56^{lck}, then this effect should be suppressed by increasing the ionic strength of assay solutions. This was the result observed and activator 1 induced stimulation of pp56^{lck} activity are decreased by high ionic strengths. The effects of ionic strength seem limited to the interactions of activator 1 and pp56^{lck} since the changes in ionic strength did not alter enzyme activities in the absence of activator 1. We have gone further to demonstrate that enzyme tyrosine phosphorylations, a known mechanism for protein kinase activation, play no role in the stimulation of pp56^{lck} by activator 1. Charge-charge interactions therefore play a major role in the interactions of pp56^{lck} with activator 1.

WORK PLAN (YEAR 3)

The above work will be continued in year 3. For the DNA binding peptides emphasis will be placed on developing assays to distinguish between specific and nonspecific DNA binding by ribonuclease chimeras containing peptides 2 and 3. In addition the effect of *lac* headpiece dimerization on its DNA binding abilities will continue to be explored. For the studies of protein-protein interactions we will synthesize additional pp56^{lck} binding peptides to isolate specific peptide structures which regulate the protein kinase. The relevance of this regulation to signal transduction across membranes by the CD4 receptor will also be explored.

PUBLICATIONS

1. A manuscript describing the pp56^{lck} work is now under preparation.

TRAINING ACTIVITIES: One graduate student has been assisting in this work.

Women or minorities -1
Non-citizens -0

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